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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: Yanagihara Case 57 U.S. APPLICATION NO.

(If known, see 37 CFR 1.5): Unknown

INTERNATIONAL APPLICATION NO.: PCT/JP99/01684 INTERNATIONAL FILING DATE: March 31, 1999

PRIORITY DATE CLAIMED: ---

TITLE OF INVENTION: PROCESS FOR FORMING AGGREGATES OF HYDROPHOBIC GROUP-CONTAINING POLYSACCHARIDE

APPLICANTS FOR DO/EO/US: (1) Ryuzo HOSOTANI, (2) Akio HAYASHI and (3) Yoshio NAKANO

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [ ] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [X] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [X] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:
    Amendment Before First Office Action
    Formal Drawings (5 sheets)
    Title Page of WIPO Document WO00/59948
    Form PCT/IB/301 – Notification of Receipt of Record Copy
    Form PCT/IB/308 – Notice Informing Applicant of Communication of International Application to Designated Offices
    International Search Report with English translation, including references with English abstracts
    Postal Card

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U.S. APPLICATION NO. (if know, see 37 CFR 1.5): Unknown

INTERNATIONAL APPLICATION NO.: PCT/JP99/01684

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17. [X] The following fees are submitted:

CALCULATIONS PTO USE ONLY

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1000.00

International preliminary examination fee (37CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... $ 860.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... $ 710.00

International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... $ 670.00

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) ... $ 100.00

ENTER APPROPRIATE BASIC FEE AMOUNT = $860.00

Surcharge of $130.00 for furnishing the oath or declaration later than [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(e)). $

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	6 - 20 =	0	X $ 18.00	$
Ind. claims	1 - 3 =	0	X $ 80.00	$
MULTIPLE DEPENDENT CLAIMS (if applicable)			+ $270.00	$

TOTAL OF ABOVE CALCULATIONS = $860.00

Reduction of 1/2 for filing by small entity, if applicable. Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28). - $

SUBTOTAL = $860.00

Processing fee of $130.00 for furnishing the English translation later than [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). + $

TOTAL NATIONAL FEE = $860.00

Fee for recording assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property + $ 40.00

TOTAL FEES ENCLOSED = $900.00

Amount to be refunded $

charged $

a. [X] A check in the amount of $900.00 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-1382. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

IN DUPLICATE

Terryence F. Chapman
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Express Mail Label No.: EL 482 000 589 US

IN THE U.S. PATENT AND TRADEMARK OFFICE

November 29, 2000

Applicants : Ryuzo HOSOTANI et al

For : PROCESS FOR FORMING AGGREGATES OF HYDROPHOBIC GROUP-CONTAINING POLYSACCHARIDE

PCT International Application No.: PCT/JP99/01684

PCT International Filing Date: March 31, 1999

U.S. Application No.
(if known, see 37 CFR 1.5): Unknown

Atty. Docket No.: Yanagihara Case 57

Box PCT
Assistant Commissioner for Patents
Washington, DC 20231

PRELIMINARY AMENDMENT CANCELING CLAIMS

Sir:

Prior to calculation of the filing fee in the above-identified application, kindly enter the following:

IN THE CLAIMS

Please amend Claims 4 and 5 as follows.

Claim 4, line 1; change "any one of claims 1" to ---Claim 1---.

line 2; delete "to 3".

Claim 5, line 1; change "any one of claims 1" to ---Claim 1---.

line 2; delete "to 4".

REMARKS

This amendment cancels claims to reduce the filing fee. Please enter this amendment before calculating the filing fee.

Respectfully submitted,

Terryence F. Chapman

TFC/smd

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Brian R. Tumm	Reg. No. 36 328

Encl: None

336.9804

09/701688

PATENT APPLICATION

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Box PCT
Assistant Commissioner for Patents
Washington, DC 20231

AMENDMENT BEFORE FIRST OFFICE ACTION

Sir:

Prior to issuance of the first Office Action in the above-identified application, kindly enter the following:

IN THE TITLE

Please change USPTO records to indicate that the title to be used in this application is ---PROCESS FOR FORMING AGGREGATES OF HYDROPHOBIC GROUP-CONTAINING POLYSACCHARIDE---, which title coincides with the title appearing in the English translation of the specification.

REMARKS

Entry of the foregoing amendment prior to issuance of the first Office Action is respectfully solicited. This amendment is intended to place the application in better form for consideration by the Examiner.

Respectfully submitted,

TFC/smd

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Brian R. Tumm	Reg. No. 36 328

Encl: None

Express Mail No. EL 482 000 589 US

5/prts

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SPECIFICATION

PROCESS FOR FORMING AGGREGATES OF HYDROPHOBIC GROUP-CONTAINING POLYSACCHARIDE

FIELD OF THE TECHNIQUE

The present invention relates to a process for forming aggregates (associated products) of hydrophobic group-containing polysaccharide.

BACKGROUND OF THE TECHNIQUE

Hydrophobic group-containing high molecular weight polysaccharides in which hydrophobic group(s) are bound to polysaccharide are used for medicinal materials, for example, coating material for coating a drug carrier enclosing therein a drug. It is known that, by coating a drug carrier, for example, liposome microcapsule, microsphere, O/W emulsion or erythrocyte ghost, with a hydrophobic group-containing polysaccharide, not only the spontaneous exudation of drug from such a drug carrier is suppressed but also the cell-specific drug transference rate using such a drug carrier is improved.

It has in recent years been widely accepted that liposome and O/W emulsion are prospective as drug carrier. It has been reported that the chemical and physical stabilities of a drug carrier of this kind within and without living body are improved by coating

the drug carrier with polysaccharide, wherein thereby a target-tropism to a specific cell group is also revealed {Bull. Chem. Soc. Japan, 62, 791 - 796 (1989)}. It has further been reported that liposomes are physically stabilized by coating them with polysaccharide {Drug Delivery System, 5, 261 (1990)}.

Further, it is reported that hydrophobic group-containing polysaccharides interact with proteins and with compounds exhibiting higher hydrophobicity so as to encapsulate these proteins or compounds {Chem. Lett., 1263 (1991)}. In this literature is described that, when aggregates of a hydrophobic group-containing polysaccharide are mixed with a globular protein of varying kind at room temperature, the protein becomes coupled with the aggregates of the hydrophobic group-containing polysaccharide to form a conjugate. Therein is also described that aggregates of hydrophobic group-containing polysaccharides are stable even in the presence of excess amounts of such proteins.

Further, a vaccine product containing a hydrophobic group-containing polysaccharide and an antigen is also known (WO 98/09650). It is furthermore known that a conjugate of a hydrophobic group-containing polysaccharide and an antigen can be isolated and purified by mixing aggregates of the hydrophobic group-containing polysaccharide with the antigen at room temperature and, then, treating the resulting mixture by gel chromatography {Macromolecules,. 7654 (1994)}.

On the other hand, Akiyoshi et al disclose in Macromolecules,. 3062 (1993) that a hydrophobicized

polymeric substances are subject to intra- or intermolecular self association of their hydrophobic groups in a dilute aqueous solution, resulting in formation of aggregates of the polymer molecules. In particular, hydrophobic group-containing polysaccharide forms relatively monodisperse microparticles of aggregates in a size of nano-order in a dilute aqueous solution by spontaneous association of several molecules. It is confirmed by observation under electron microscope that relatively monodisperse globular microparticles of nano-order size are formed. Such relatively monodisperse nano-order size aggregates of hydrophobic group-containing polysaccharide exist in water as a dispersion which is colorless and transparent in appearance and does not form any cloud or precipitate even after standing still for a long period of time, leaving an appearance of aqueous solution in human eye.

By causing a hydrophobic group-containing polysaccharide to swell in water and agitating the resulting swollen dispersion using, for example, a homomixer, a turbid dispersion is obtained. In such a turbid dispersion, a part of the hydrophobic group-containing polysaccharide forms aggregates of a size of nano-order, while there are at the same time some which are present as lumps of various sizes without forming such aggregates. When a turbid liquid, in which lumps of sizes greater than nano-order size are present, is used for a medicinal material, for example, as a material in a drug delivery system (DDS) for intravenous administration, thrombus may be formed due to the

above-mentioned lumps. When, on the other hand, the colorless transparent liquid in which the hydrophobic group-containing polysaccharide is present, forming aggregates of uniform nano-order size, is used therefor, there is no fear of thrombus formation. Therefore, there is a demand for aggregates of hydrophobic group-containing polysaccharide which are dissolved (dispersed in a colorless transparent state) in water, in order to use the hydrophobic group-containing polysaccharide as, for example, a medicinal material for building up a conjugate with a varying kind of drug or protein.

In the past, processes have been known for forming hydrophobic group-containing polysaccharide into aggregates, for example, 1) a process in which the hydrophobic group-containing polysaccharide is dissolved in dimethyl sulfoxide (DMSO) under a dilute condition and the resulting solution is then dialyzed against water and 2) a process in which the hydrophobic group-containing polysaccharide is caused to swell in water and the resulting swollen dispersion is then treated by ultrasonication {Macromolecules,. 3062 (1993); WO 98/09650}.

It is, however, quite difficult to prepare such aggregates of hydrophobic group-containing polysaccharide industrially in large scale by the above-mentioned processes of prior art. Firstly, for example, in the dialysis process of the prior art, there are problems in that 1) a dialysis arrangement capable of large scale treatment is required, 2) a huge amount of water is necessary and 3) a prolonged period of time

is required for the treatment. Secondly, in the process by ultrasonication of the prior art, there are problems in that 1) the throughput of one single treatment is lower, 2) deviation between treating lots is large, since control of sonication efficiency and of sonication time is difficult and monodisperse aggregates are not able to obtain steadily and 3) probable contamination with, for example, fractured metal fragments occurred due to deterioration of the sonication tip may occur.

On the background described as above, a large scale preparation of aggregates of hydrophobic group-containing polysaccharide is difficult by the processes with dialysis and ultrasonication of the prior art. Therefore, a more simple and convenient process for forming aggregates of hydrophobic group-containing polysaccharide is expected. However, no technique of forming aggregates of hydrophobic group-containing polysaccharide has hitherto been known other than the above-mentioned processes of dialysis and ultrasonication.

While a homogenizer is used for emulsifying oils in water, no practical experience has heretofore been known in which a homogenizer is used for forming aggregates of hydrophobic group-containing polysaccharide.

The object of the present invention is to obviate the problems in the prior art described above and to provide a process for forming aggregates of hydrophobic group-containing polysaccharide, in which

the deviation between treating lots and the contamination by impurities are eliminated and which can afford to prepare uniform aggregates of hydrophobic group-containing polysaccharide steadily in a simple and convenient way within a brief time in large scale.

DISCLOSURE OF THE INVENTION

The inventors reached from their sound researches a knowledge that aggregates of a hydrophobic group-containing polysaccharide can be obtained in a simple and convenient way within a brief time in large scale by dispersing a swollen liquor of the hydrophobic group-containing polysaccharide using a high pressure homogenizer, whereby the present invention has been completed. Thus, the present invention consists in the process for forming aggregates of hydrophobic group-containing polysaccharide as given below:

(1) A process for forming aggregates of hydrophobic group-containing polysaccharide in water, comprising

causing the hydrophobic group-containing polysaccharide to swell in water and treating the resulting swollen dispersion by dispersing it using a homogenizer under a pressure of 9.8 - 490 MPa (100 - 5,000 kgf/cm^2).

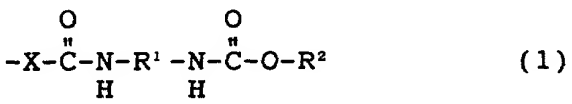
(2) The process as defined in the above (1), wherein the homogenizer is a high pressure homogenizer.

(3) The process as defined in the above (1), wherein the homogenizer is a high pressure homogenizer which operates so as to jet the swollen dispersion pressurized

under a pressure of 9.8 - 490 MPa (100 - 5,000 kgf/cm^2) into a chamber from an orifice to disperse the swollen dispersion to treat it.

(4) The process as defined in any one of the above (1) to (3), wherein the aggregates of the hydrophobic group-containing polysaccharide have particle sizes of 10 - 30 nm and numbers of associations of the hydrophobic group-containing polysaccharide molecules of 3 - 20.

(5) The process as defined in any one of the above (1) to (4), wherein the hydrophobic group-containing polysaccharide has -XH groups (wherein X denotes oxygen atom or a nitrogen-containing group represented by NY with Y standing for hydrogen atom or a hydrocarbon group of 1 - 10 carbon atoms), wherein 0.1 - 10 -XH groups per 100 monosaccharide units constituting the polysaccharide are replaced by one or more hydrophobic groups represented by the formula (1), namely,



$$-X-\overset{\overset{O}{\|}}{C}-\underset{H}{N}-R^1-\underset{H}{N}-\overset{\overset{O}{\|}}{C}-O-R^2 \qquad (1)$$

in which X is the same as given above, R^1 denotes a hydrocarbon group having 1 - 50 carbon atoms and R^2 denotes a hydrocarbon group of 12 - 50 carbon atoms or a steryl group.

(6) The process as defined in the above (5), wherein the polysaccharide to be substituted by hydrophobic group(s) consists of any one selected from the group consisting of pullulan, amylopectin, amylose, dextran, hydroxyethyl cellulose, hydroxyethyl dextran,

mannan, levan, inulin, chitin, chitosan, xyloglucan and water-soluble cellulose.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the results of Example 1-1 in graphs, each in a chart of the results of SEC analyses of a pullulan-cholesterol derivative (CHP) before and after the treatment by a high pressure homogenizer. Figs. 1(a) and 1(b) are each a chart of analysis result of SEC before and after the treatment of the CHP by the high pressure homogenizer, respectively. The ordinate represents the strength (dimensionless) of the differential refractometer (the same applies to those in the following).

Fig. 2 shows the results of Examples 1-2 to 1-5 in graphs, wherein Figs. 2(a), 2(b), 2(c) and 2(d) are each a chart of analytical result of SEC after the treatment by high pressure homogenizer for Examples 1-2, 1-3, 1-4 and 1-5, respectively.

Fig. 3 shows the result of Comparative Example 1 in a graph, a chart of the result of SEC analysis after the dialysis.

Fig. 4 shows by charts of results of SEC analyses of pullulan (of a molecular weight of 108,000) and of CHP. Figs. 4(a), 4(b) and 4(c) are each a chart of the result of SEC analysis, for the pullulan (molecular weight 108,000), for the CHP and for the aggregates of the CHP, respectively.

Fig. 5 is a chart of the result of SEC analysis

of CHP (a concentration of 0.2 % by weight) after an ultrasonication for a predetermined period of time.

Fig. 6 shows the results of Comparative Example 2 in graphs, namely, charts of the SEC analysis results of a CHP after an ultrasonication treatment and after a treatment by a high-pressure homogenizer, respectively. Figs. 6(a) is a chart of the result of SEC analysis of the CHP after the ultrasonication treatment. Fig. 6(b) is a chart of the result of SEC analysis of the ultrasonicated liquor of Fig. 6(a) after it is treated by the high-pressure homogenizer.

THE BEST MODE FOR EMBODYING THE INVENTION

While there is no special limitation for the hydrophobic group-containing polysaccharide to be employed according to the present invention, so long as it has hydrophobic groups, the following hydrophobic group-containing polysaccharides are preferred. Thus, preference is given to polysaccharides having -XH groups (wherein X denotes oxygen atom or a nitrogen-containing group represented by NY with Y standing for hydrogen atom or a hydrocarbon group of 1 - 10 carbon atoms), wherein 0.1 - 10, preferably 0.1 - 6, -XH groups per 100 monosaccharide units constituting the polysaccharide are replaced by one or more hydrophobic groups represented by the formula (1) given above.

As the hydrocarbon group having 1 - 50 carbon atoms represented by R^1 in the above formula (1), there may be enumerated, for example, radicals of ethylene,

butylene, hexamethylene and diphenylmethane.

As the hydrocarbon group having 12 - 50 carbon atoms or the steryl group represented by R^2 in the above formula (1), there may be enumerated, for example, laulyl, myristyl, cetyl, stearyl, cholesteryl, stigmasteryl, β -sitosteryl, lanosteryl and ergosteryl.

For the polysaccharide to be substituted by hydrophobic groups (in the following, denoted sometimes as the pre-substitution polysaccharide) represented by the formula (1) given above for the hydrophobic group-containing polysaccharide, those of natural occurrence and semisynthetic ones may be exemplified. As preferred pre-substitution polysaccharide, there may be exemplified one or more of those selected from the group consisting of pullulan, amylopectin, amylose, dextran, hydroxyethyl cellulose, hydroxyethyl dextran, mannan, levan, inulin, chitin, chitosan, xyloglucan and water-soluble cellulose. Among them, pullulan, mannan, xyloglucan, amylopectin, amylose, dextran and hydroxyethyl cellulose are preferred. Polysaccharides having nitrogen atom(s), such as chitin, partially deacetylated chitin and chitosan, are also favorable. The polysaccharides may be employed either alone or in a combination of two or more of them.

The hydrophobic group-containing polysaccharide having the hydrophobic groups represented by the above formula (1) can be produced by a known technique. For example, it can be produced by a method, in which a diisocyanate compound represented by the formula $OCN-R^1-NCO$ (in which R^1 denotes a hydrocarbon group

having 1 - 50 carbon atoms) is reacted with a hydroxyl group-containing hydrocarbon having 12 - 50 carbon atoms or a sterol represented by the formula R^2-OH (in which R^2 denotes a hydrocarbon group having 12 - 50 carbon atoms or a steryl group) in the first reaction step in which one mole of the hydroxyl group-containing hydrocarbon having 12 - 50 carbon atoms or the sterol is reacted to form an isocyanato group-containing hydrophobic compound, whereupon, in the second reaction step, the isocyanato group-containing hydrophobic compound obtained in the first reaction step is reacted with the pre-substitution polysaccharide mentioned above.

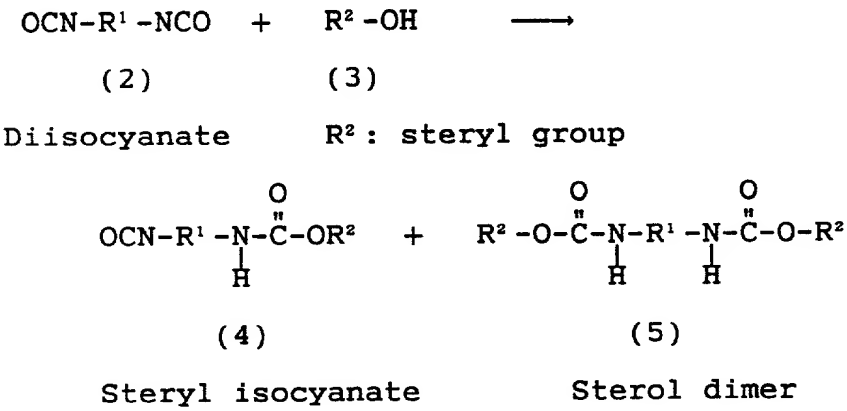
Concrete examples of the diisocyanate compound (OCN-R^1-NCO) to be used in the first reaction step include ethylene diisocyanate, butylene diisocyanate, hexamethylene diisocyanate and diphenylmethane diisocyanate, namely, those in which R^1 is a radical of ethylene, butylene, hexamethylene and diphenylmethane, respectively.

As the hydroxyl group-containing hydrocarbon (R^2-OH) having 12 - 50 carbon atoms to be used in the first reaction step, there may be enumerated, for example, those originated from alcohols, such as lauryl alcohol, myristyl alcohol, cetyl alcohol, stearyl alcohol, arachidic alcohol, docosanol, pentacosanol, hexacosanol and octacosanol. Among them, those having 12 - 35 carbon atoms, in particular, those having 12 - 20 carbon atoms are preferred because of their easy availability. As the sterol (R^2-OH), there may be

enumerated, for example, cholesterol, stigmasterol, β-sitosterol, lanosterol and ergosterol.

An example of the second step reaction is shown below by the reaction schemes (I) and (II). In the reaction schemes given below, pullulan is employed as the pre-substitution polysaccharide. In the reaction scheme (I), a diisocyanate compound represented by the formula (2) is reacted with a sterol represented by the formula (3) to form the stearyl isocyanate represented by the formula (4). In this reaction, usually sterol dimer represented by the formula (5) is formed as a by-product. In the reaction scheme (II), the stearyl isocyanate represented by the formula (4) obtained by the reaction scheme (I) is reacted with pullulan represented by the formula (6) to produce a polysaccharide-sterol derivative (hydrophobic group-containing polysaccharide) represented by the formula (7).

Reaction Scheme (I)



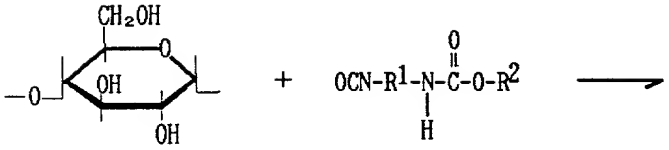
(2) (3)

Diisocyanate R^2: steryl group

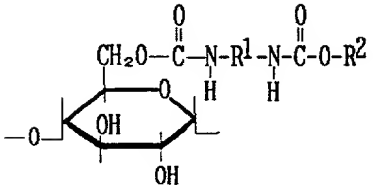
(4) (5)

Steryl isocyanate Sterol dimer

Reaction Scheme (II)



(6) (4)



(7) (Polysaccharide-sterol derivative)

The hydrophobic group-containing polysaccharide obtained by the above reactions can be purified by means of a known technique, such as precipitation or centrifugation. By the purification, the sterol dimer (by-product) represented by the formula (5) can be removed. The hydrophobic group-containing polysaccharide having hydrophobic groups represented by the formula (1) and the production process thereof are given in detail in, for example, Japanese Patent Kokai Hei 3-292301 A and in Macromolecules,. 3062 (1993).

The process for forming the aggregates according to the present invention can be realized by the process steps [1] and [2] given in the following.

Process step [1]

The hydrophobic group-containing polysaccharide is caused to swell in water.

Process step [2]

The swollen dispersion obtained in the above process step [1] is subjected to a dispersing treatment

using a homogenizer under a pressure of 9.8 - 490 MPa (100 - 5,000 kgf/cm^2). The dispersing treatment of the process step [2] may be effected in two or more repeats. By repeating several times, the state of dispersion of the aggregates becomes more stable.

Below, the process for forming according to the present invention will further be described in more detail.

The amount of water to be used in the process step [1] may favorably be 30 - 10,000 times weight, preferably 100 - 1,000 times weight of the hydrophobic group-containing polysaccharide. If this amount is short of 30 times weight, the hydrophobic group-containing polysaccharide may become unfavorable gelled state. If this amount exceeds over 10,000 times weight, the efficiency of forming aggregates will become unfavorably decreased. While there is no special restriction as to the water temperature for effecting swelling, a temperature of 0 - 100 °C, preferably 10 - 50 °C, may be favorable.

The resulting swollen dispersion may favorably be brought to the subsequent process step [2] after having been stirred by a stirrer. As the stirrer to be employed, a magnetic stirrer, a homomixer or the like may be exemplified. Among them, preference is given to homomixer. While there is no special limitation for the revolution rate, stirring duration and so on of the stirrer, a revolution rate of 100 - 15,000 rpm and a stirring duration of 30 seconds to 180 minutes may be favorable. The dispersion resulting from stirring of

the swollen dispersion is present as a turbid liquid, which gives birth to deposition of precipitate after standing for a while.

The homogenizer to be employed in the process step [2] should be capable of dispersion-treating the swollen dispersion from the process step [1] under a pressure of 9.8 - 490 MPa (100 - 5,000 kgf/cm^2), preferably 98 - 294 MPa (1,000 - 3,000 kgf/cm^2). For such a homogenizer, commercial high pressure homogenizer may be employed. A high pressure homogenizer is a device for attaining emulsification or microdispersion of a liquor by generating shearing forces, impingement momentums and cavitation by the aid of a high pressure.

When such a high pressure homogenizer is used, aggregates of the hydrophobic group-containing polysaccharide can be formed, concretely, in the following manner. First, the swollen dispersion is pressurized at a pressure mentioned above and the so-pressurized swollen dispersion is spouted from an orifice into a chamber to cause cavitation (pressure drop). The spouted swollen dispersion is thereby accelerated and is caused to bring about intense collisions of domains of the swollen dispersion with each other in the chamber and with the walls of the chamber. By the thereby generated impingement momentums and shearing forces, the hydrophobic group-containing polysaccharide is dispersed finely in the dispersion to build up aggregates thereof. The so-obtained treated liquor is present as a transparent colorless liquid which is a dispersion (expressed in the following

sometimes as aqueous solution) not subject to occurrence of turbidity or precipitation after a prolonged standing still.

The dispersing treatment using high pressure homogenizer may be effected only once or in two or more repeats. The treatment with high pressure homogenizer may be carried out in a batchwise or continuous operation. While the number of repeats of the high pressure homogenizer treatment may vary considerably depending on, for example, each specific hydrophobic group-containing polysaccharide, the degree of substitution with such hydrophobic group, the concentration in the aqueous dispersion and the pressure on the high pressure homogenizer treatment, a stable and relatively monodisperse aggregate may be obtained usually by five repeats, though not affirmable. For example, in the case where the hydrophobic group-containing polysaccharide is a pullulan-cholesterol derivative with a cholesterol-substitution degree of 1.2 cholesterol groups per 100 monosaccharide units, the concentration in the aqueous dispersion is 0.2 % by weight and the pressure on the high pressure homogenizer treatment is 98 MPa (1,000 kgf/cm^2), a stable aggregate without suffering from occurrence of turbidity or precipitation can be obtained by repeating the dispersing treatment by the high pressure homogenizer three times.

Concrete examples of the high pressure homogenizer which can be used in the process according to the present invention include MICROFLUIDIZER (of the

firm Microfluidex, trademark), MICROFLUIDIZER (of Mizuho Kogyo K.K., trademark), DeBEE 2000 (trademark, supplied from Q.P. Corp.) and APV GAULIN (trademark, of APV Rannie, Inc.).

While there is no special limitation as to the temperature of the swollen dispersion on the dispersing treatment by a homogenizer, a temperature in the range from 0 to 100 °C, preferably from 10 to 50 °C, may be favorable.

By performing the dispersing treatment using a homogenizer, a monodisperse aggregate can be formed. The resulting monodisperse aggregate, namely, the aggregate of the hydrophobic group-containing polysaccharide obtained by the process according to the present invention, has usually an aggregate particle size in the range from 10 to 30 nm and a number of associations of the hydrophobic group-containing polysaccharide in the aggregate in the range from 3 to 20. Here, the particle size and the number of associations refer both to the average value. The resulting treated dispersion is a colorless transparent aqueous solution which will not become turbid nor bring about precipitation after a prolonged standing still. Here, the monodisperse aggregate will not be formed by simply agitating the swollen dispersion by a stirrer, such as a magnetic stirrer or homomixer. The swollen dispersion keeps its turbid state and will not turn into colorless transparent state even though the revolution rate of the stirrer is increased or the stirring is continued for prolonged period of time.

The aggregate of the hydrophobic group-containing polysaccharide formed by the process according to the present invention can be separated in a form of a solid matter by drying the aggregate, after it has been formed, by means of, for example, freeze-drying. From this solid matter, a colorless transparent aqueous solution of the aggregate in the state before the freeze-drying can be restored by adding water to the solid matter.

The aggregate of the hydrophobic group-containing polysaccharide formed by the process according to the present invention can be used as a medicinal material, such as a coating material for coating a drug carrier enclosing therein a drug. Thus, it can be used as a coating material for coating a drug carrier made of, for example, liposome, microcapsule, microsphere, O/W emulsion or erythrocyte ghost. Here, the aggregate of the hydrophobic group-containing polysaccharide obtained by the process according to the present invention can be used securely as a medicinal material and for preparing a drug carrier of stable quality, since the so-obtained aggregate is a homogeneous product and has no quality deviation between production lots nor contamination by impurity. The aggregate of hydrophobic group-containing polysaccharide obtained by the process according to the present invention can also be utilized as surfactants, thickening agents and a raw material for cosmetics.

In the process according to the present

invention, it is possible to employ a mixture of hydrophobic group-containing polysaccharide with one or more polysaccharides having no hydrophobic group (i.e. those before introduction of hydrophobic group therein) and/or one or more medicaments and/or one or more proteins, instead of using the hydrophobic group-containing polysaccharide solely. Hereby, the possibility of extension of application field, for example, in the drug delivery system (DDS), may be prospective.

As described above, aggregates of hydrophobic group-containing polysaccharide can securely be formed in a homogeneous quality steadily within a brief period of time, in a large scale and in a simple manner, by the process according to the present invention without suffering from quality deviation between production lots and from contamination by impurity, since the process is performed by a dispersing treatment of a swollen dispersion of the hydrophobic group-containing polysaccharide using a homogenizer under a pressure within a specific range.

Below, the present invention will concretely be described by way of Examples, though these Examples should not be regarded as restricting the scope of the present invention.

In all the Examples, the experimental conditions employed were as follows:

《 Conditions of Size Exclusion Chromatography (SEC) 》

1) Apparatus used: TOSOH HPSEC SYSTEM (trademark, of Tosoh Ltd.)

2) Column: TSK-gel G4000SWXL (trademark, of Tosoh Ltd.)

3) Eluent: 0.05 % NaN_3 in deionized water

4) Flow rate: 0.5 ml/min.

5) Temperature: 35 °C

6) Detector: RI (a differential refractometer)

《 Determination of Particle Size by Dynamic Light-Scattering Measurements 》

Apparatus used: DLC-700 (trademark, of Otsuka Electronics Co., Ltd.)

Conditions of Determination: 5 mW He-Ne laser (633 nm); temperature = 25 °C; scattering angle = 25°; concentration = 4.15 mg/ml

《 Determination of Number of Assocoations by Static Light-Scattering Measurements 》

Apparatus used: DLC-700 (trademark, of Otsuka Electronics Co., Ltd.)

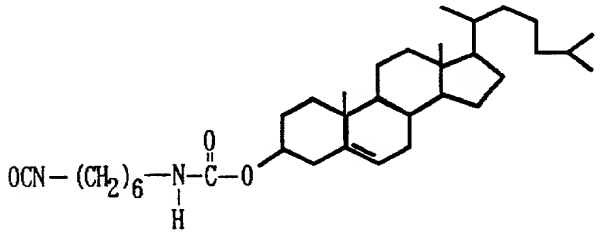
Conditions of Determination: MR-102 (differential refractometer); temperature = 25 °C; scattering angle = 30° - 130°; concentration = 0.72 - 1.93 mg/ml

Synthesis Example 1-1

《 Synthesis of N-(6-isocyanatohexyl)cholesteryl carbamate 》

An eggplant type 1-liter flask was charged with 25 grams (0.065 mol) of cholesterol and thereto were added 300 ml of toluene to dissolve it, whereto 17 ml (0.12 mol) of triethylamine were added. To this, 161 grams (0.96 mole, 14.8 eq.) of hexamethylene diisocyanate dissolved in 300 ml of toluene were added to cause a reaction at 80 °C for 6 hours under a

nitrogen atmosphere. After termination of the reaction, toluene and the excess amount of hexamethylene diisocyanate were removed by reducing the pressure. The resulting yellowish oily residue was stood still overnight at room temperature to cause precipitation of pale yellow crystals. The crystals were taken out and about one liter of hexane was added thereto, whereupon the mixture was shaken vigorously and, then, the supernatant liquid was removed by decantation. This washing procedure was repeated four times, whereupon the crystals were dried under a reduced pressure at room temperature for three hours, whereby N-(6-isocyanatohexyl)cholesteryl carbamate represented by the following formula (4a) was obtained.



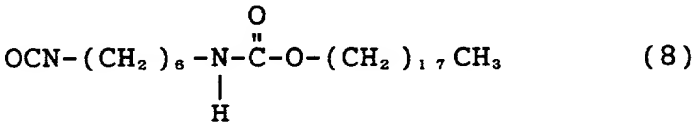
(4a)

Synthesis Example 1-2

《 Synthesis of N-(6-isocyanatohexyl)stearyl carbamate 》

In an eggplant type flask of 300 ml capacity, there were charged 3.48 g (12.9 mmol) of stearyl alcohol and thereto were added 50 ml of toluene to dissolve it, whereto 2.04 g (25.8 mmol) of pyridine were further added. To this mixture, there were added 30 g (178 mmol, 14.8 eq.) of hexamethylene diisocyanate dissolved

in 50 ml of toluene and the resulting mixture was subjected to reaction at 80 °C under a nitrogen atmosphere for about 3 hours. After termination of the reaction, toluene and the excess of hexamethylene diisocyanate were removed under a reduced pressure, whereby a pale yellow crystals were formed. The crystals were taken out, whereto about one liter of hexane was added and the mixture was shaken vigorously, whereupon the supernatant was removed by decantation. This washing procedure was repeated four times, whereupon the product was dried under a reduced pressure for three hours at room temperature. Hereby N-(6-isocyanatohexyl)stearyl carbamate represented by the following formula (8) was obtained:



$$OCN-(CH_2)_6-N(H)-C(=O)-O-(CH_2)_{17}CH_3 \qquad (8)$$

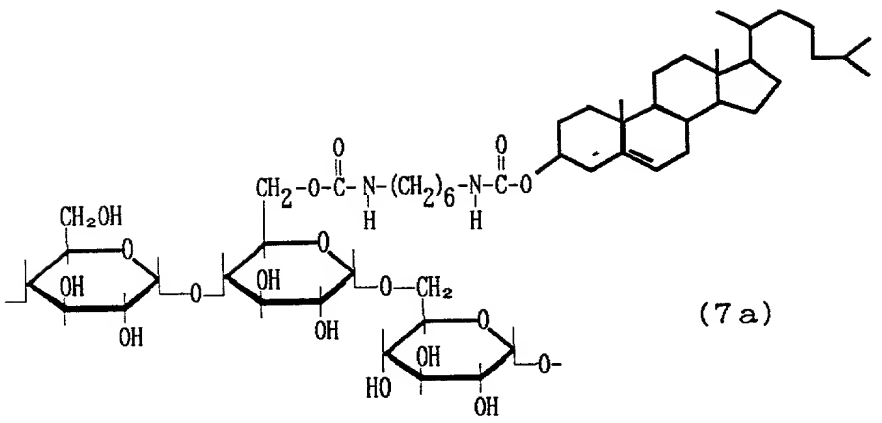
Synthesis Example 2

《 Synthesis of pullulan-cholesterol derivative (CHP) 》

A hydrophobic group-containing polysaccharide was synthesized according to the method of Akiyoshi et al {Macromolecules,. 3062 (1993)}. Thus, an eggplant type flask of 1 liter capacity was charged with 40 g (248 mmol as anhydrous glucose unit) of a pullulan (a product of Wako Pure Chemical Industries, Ltd.; average molecular weight: 108,000) and 420 ml of dimethyl sulfoxide (sometimes abbreviated as DMSO) and the mixture was agitated at 80 °C under a nitrogen atmosphere to dissolve it. To this solution, a solution

of 1.78 g (3.21 mmol) of N-(6-isocyanatohexyl)cholesteryl carbamate synthesized in Synthesis Example 1-1 dissolved in 32.4 ml (0.40 mol) of pyridine was added and the mixture was subjected to reaction at 90 °C for 1.5 hours.

After termiantion of the reaction, dimethyl sulfoxide was removed by reducing the pressure and the resulting oily residue was dropped into 6 liters of acetone to form a precipitate which was purified. After removal of the supernatant, 4 liters of acetone were added to the resulting precipitate and the mixture was stood still overnight at room temperature. The precipitate was collected by filtration and was dried under a reduced pressure. The so-obtained solids were dissolved in dimethyl sulfoxide and the solution was charged in a dialysis bag (Spectra/Por3, a product of the frim Spectropor; a fractionating molecular weight of 3,500) and was subjected to a dialysis against distilled water for one week. 1.5 liters of the resulting polymer solution were treated by freeze-drying in an ordinary manner, whereby a pullulan-cholesterol derivative (abbreviated hereinafter sometimes as CHP) represented by the following formula (7a) was obtained. By calculating the proportion of introduction of the cholesteryl groups into the pullulan in the CHP from the integration value of ^{1}H-NMR spectrogram of CHP, it was determined that the proportion of substitution with cholesteryl group in the pullulan-cholesterol derivative (CHP) represented by the formula (7a) was about 1.3 groups per 100 monosaccharide units.



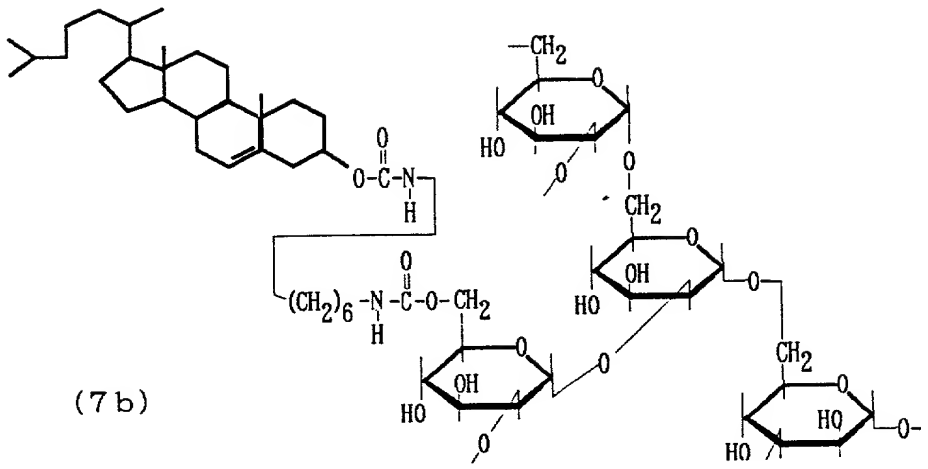
(7a)

Synthesis Example 3

By the procedures similar to those in Synthesis Example 2, a pullulan-cholesterol derivative (CHP), in which about 2.8 cholesteryl groups are introduced per 100 monosaccharide units, was synthesized.

Synthesis Example 4

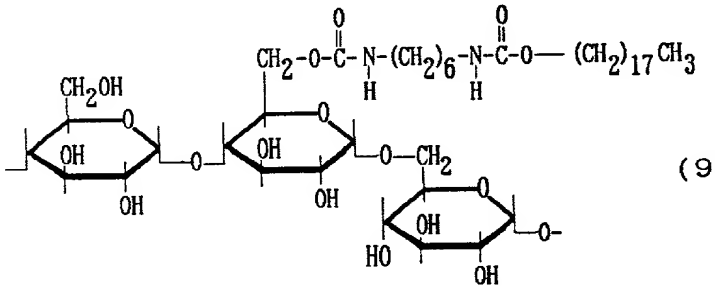
In the same manner as in Synthesis Example 2, except that a commercial mannan (a produact of the firm Sigma) having an average molecular weight of about 85,000 was used in the place of the pullulan, a mannan-cholesterol derivative (in the following, sometimes abbreviated as CHM), in which about 2.3 cholesteryl groups are introduced per 100 monosaccharide units, represented by the following formula (7b) was synthesized.



(7b)

Synthesis Example 5

In the same manner as in Synthesis Example 2, except that N-(6-isocyanatohexyl)stearyl carbamate synthesized in Synthesis Example 1-2 was used in the place of N-(6-isocyanatohexyl)cholesteryl carbamate synthesized in Synthesis Example 1-1, a stearylpullulan (in the following, sometimes abbreviated as STP), in which about 0.8 stearyl group was introduced per 100 monosaccharide units, represented by the following formula (9) was synthesized.



(9)

Example 1-1

There were added 1,000 ml of water to 2 grams of the CHP obtained in Synthesis Example 2 to cause the CHP to swell at a temperature of 60 °C for 2 hours (CHP

concentration = 0.2 % by weight). The resulting swollen dispersion was then stirred using a homomixer (5,000 r.p.m.) for 5 minutes. The appearance of the dispersion at this occasion was white turbid. The so-stirred swollen dispersion of 20 °C was subjected to a homogenization by causing the dispersion to spout out of an orifice under a pressure of 98 MPa (1,000 kgf/cm^2) using MICROFLUIDIZER (trademark, a high pressure homogenizer Model M-110Y of the firm Mizuho Kogyo K.K.) into a chamber in order to disperse it. This homogenization treatment was repeated twice. The herein used MICROFLUIDIZER had a treating capacity of about 500 ml/min. and the time required for the twice repeats of the homogenization treatment was about 5 minutes. The resulting treated liquor had a colorless and transparent appearance. For this aqueous solution, the particle size and the number of associations were determined by the methods indicated above. The results are summarized in Tables 1 and 2.

The above aqueous solution was analyzed also by a size-exclusion chromatography (SEC). The results obtained for the solution before the treatment by the high pressure homogenizer are shown in Fig. 1(a) and those after the treatment are shown in Fig. 1(b). From the results as given in Figs. 1(a) and 1(b), it was confirmed that aggregates of the CHP were formed by treating the swollen dispersion by the high pressure homogenizer.

Then, the resulting aqueous solution of the CHP aggregates was subjected to a freeze-drying, whereby

the aggregates of the CHP were isolated as a white solid matter. To this solid matter, water was added so that a concentration of 0.2 % by weight would be reached, whereupon the mixture was stood still at room temperature for three hours in order to restore an aqueous solution. The restored solution was colorless and transparent. For the aqueous solution of the CHP aggregates before the freeze-drying and for the restored solution, SEC analyses were carried out, whereby it was recognized that there was no distinction in the chart curve between both the solutions and was confirmed that both are identical.

Examples 1-2 to 1-6

By the same procedures as in Example 1-1, homogenization treatments were carried out using the hydrophobic group-containing polysaccharides and under the conditions as recited in Table 1. The results are summarized in Tables 1 and 2. The results of SEC analysis are shown in Figs. 2(a) to 2(d). From the results as shown in Figs. 2(a) to 2(d), it was confirmed that all the Examples showed formation of the aggregate of the hydrophobic group-containing polysaccharide.

By performing the freeze-drying in the same manner as in Example 1-1, the aggregates in each Example were isolated in a form of white solid matter. For the aqueous solution of the aggregates before and after the freeze-drying, comparison was carried out as in Example 1-1, whereby it was recognized that there was no distinction therebetween and was confirmed that both are identical.

Table 1 Hydrophobic group-containing polysaccharide

	Example					
	1-1	1-2	1-3	1-4	1-5	1-6
Hydropho. group-containg polysaccharide	Synthesis Example 2	Synthesis Example 2	Synthesis Example 3	Synthesis Example 4	Synthesis Example 5	Synthesis Example 2
Abbrev. of h.p.s. *)	CHP	CHP	CHP	CHM	STP	CHP
Starting polysaccharide	Pullulan	Pullulan	Pullulan	Mannan	Pullulan	Pullulan
Hydrophobic group	Cholester.	Cholester.	Cholester.	Cholester.	Stearyl	Cholester.
Introduct. proportion of hydrophobic group 1)	1.3	1.3	2.8	2.3	0.8	1.3
Content of unreacted polysaccharide (wt. %)	0	0	0	0	0	13
Cont. of dimer (wt.%) 2)	0	0	0	0	0	0
Purity (wt.%) 3)	100	100	100	100	100	87

Notes: *) Abbreviation of the hyrophobic-group-containing polysaccharide.

1) Number of the introduced groups per 100 monosaccharide units.

2) Content of the by-products resulting from reaction of two NCO-groups in the diisocyanate in Synthetic Example 1-1 or 1-2.

3) Purity of the hydrophobic group-containing polysaccharide.

Table 2 Homogenizer treatment and the results

	Example					
	1-1	1-2	1-3	1-4	1-5	1-6
Polysacchar. of Table 1						
Amount used (g)	2	5	10	20	2	5
Concentration (wt. %)	0.2	0.5	0.2	0.5	0.2	0.5
Treatment						
Amt. of dispersion (ml)	1,000	1,000	500	400	1,000	1,000
Treat. pressure (MPa)	98	196	294	98	108	196
Repeats (times)	2	3	5	3	2	3
Duration (min.)	5	8	12	8	5	5
Mw ($\times 10^5$) *1)	1.53	1.61	1.49	1.80	1.30	1.61
Mw/Mn *2)	1.06	1.06	1.12	1.08	1.09	1.06
Content of unreacted polysaccharide (wt. %)	0	0	0	0	0	13
Aggregates						
Particle size (nm)	20	20	16	18	22	20
Number of assoc.	6	8	7	7	10	8

Note: *1) Mw: Weight-average molecular weight.

*2) Mw/Mn: Molecular weight distribution; Mn = number average molecular weight

Comparative Example 1

《Formation of Aggregate by Dialysis》

Two grams of the CHP obtained in Synthesis Example 2 were dissolved in 100 ml of dimethyl sulfoxied (DMSO). The resulting solution was charged in a dialysing bag (Spectra/Por3, supplied from the firm Spectrum; fractionating molecular weight: 3500) and was dialysed against distilled water for four days. The results of SEC analysis of the resulting dialysed liquor are shown in Fig. 3. From the results shown in Fig. 3, it is seen that monodisperse aggregates were not obtained.

As examples of aggregates of the hydrophobic group-containing polysaccharide, results of SEC analyses are shown in Figs. 4(a), 4(b) and 4(c), which were performed (a) for a pullulan having a molecular weight of 108,000; (b) for a water-dispersion of a pullulan-cholesterol derivative (CHP) based on the above pullulan (1.3 cholesteryl groups are introduced per 100 monosaccharide units of the pullulan); and (c) for the above CHP after ultrasonic wave treament after having been dispersed in water, respectively.

An elution peak is recognized for the CHP on the side of higher molecular weight than that of the pullulan, indicating occurrence of an intermolecular association. It is also seen from Figs. 4(b) and 4(c) that the CHP which was in a relatively loose association state in the dispersion was brought into formation of relatively monodisperse aggregates by the ultrasonic wave treatment. Calculation of the apparent degree of

dispersion of the aggregates shown in Fig. 4(c) by reference to a standard sample of pullulan gave an Mw/Mn value of 1.1. By performing the determination by the above light-scattering for these aggregates, it has been detected that they are microparticles having particle sizes of 15 - 25 nm in which about 8 molecules are in association.

Comparative Example 2

《 Formation of Aggregate by Ultrasonication 》

In the same manner as in Example 1-1, two grams of the CHP obtained in Synthetic Example 2 were subjected to swelling at 60 °C for two hours by introducing thereinto 1,000 ml of water (CHP concentration = 0.2 % by weight). This swollen dispersion was treated by ultrasonication using a probe-type sonicator (made of the firm Nippon Seiki; external diameter of the probe = 12 mm) at 150 W for 60 minutes. During the ultrasonication, the treating vessel was cooled from outside with ice-water to maintain the dispersion temperature always at 4 °C or lower. At each predetermined point of time (0, 3, 5, 10, 15, 30 and 60 minute) a sample of the dispersion was taken, for which SEC analyses were carried out for observing temporal variation in the formation of the aggregate. The results of SEC analyses at each occasion are shown in Fig. 5.

From the results shown in Fig. 5, it is seen that formation of aggregate was effected as the time elapsed. In the elution curve, a shoulder is seen even after 60 minutes, whereby it can be confirmed that

monodisperse aggregates were not formed. When the ultrasonication was extended for further 60 minutes, the shoulder of the elution peak was recognized and no variation was seen. By analyzing this sample by the above dynamic light-scattering, it was observed that the particle size was about 128 nm.

Reference Example 1

A swollen dispersion of a concentration of 0.5 % by weight of the CHP obtained in Synthesis Example 2 was prepared, which was analyzed by SEC after having been subjected to an ultrasonication under the same condition as in Comparative Example 2 for 60 minutes {Fig. 6(a)}. Here, it was shown that the form of the peak is complicated and formation of aggregate is insufficient. Therefore, it is recognizable that the effect of ultrasonication depends on the concentration. When the ultrasonication was extended for further 60 minutes, no change in the form of the peak was recognized. This ultrasonicated dispersion which showed insufficient formation of aggregate was treated using the MICROFLUIDIZER mentioned above {98 MPa (1,000 kgf/cm^2); no repeat of treatment}. The results of SEC analysis of the so-treated liquor were as shown in Fig. 6(b). By further analyses by the above dynamic light-scattering and static light-scattering, it was confirmed that the particle size was about 18 nm and the number of molecules in association were about 8.

From the results given above, it is seen that a sufficient formation of aggregate was not able to attain using an ultrasonication, whereas the process as

shown in Examples using a high pressure homogenizer was able to attain formation of aggregate easily.

It is also seen that aggregates exhibiting a narrower molecular weight distribution were formed in Examples 1-1 to 1-6 in which a high pressure homogenizer was used, as compared with the results of Comparative Example 1 in which dialysis was employed and of Comparative Example 2 in which an ultrasonic wave treatment was used. It is further seen that aggregates of a hydrophobic group-containing polysaccharide can be formed within a brief time in a simple and convenient manner in large amount by the process according to the present invention, since inventive Example 1-1 showed a productivity of 2 grams in a treating time of 5 minutes, whereas Comparative Example 1 using dialysis showed a productivity of 2 grams in a treating time of 4 days and Comparative Example 2 using ultrasonication showed a productivity of 2 grams in a treating time of more than two hours.

INDUSTRIAL APPLICABILITY

The aggregates of hydrophobic group-containing polysaccharide formed by the process according to the pesent invention can be utilized as a coating material for coating drug carriers encapsulating therein drugs. For example, it can be used as the coating material for coating drug carriers, such as liposome microcapsules, microspheres, O/W emulsions and erythrocyte ghost.

CLAIMS

1. A process for forming aggregates of hydrophobic group-containing polysaccharide in water, comprising

causing the hydrophobic group-containing polysaccharide to swell in water and treating the resulting swollen dispersion by dispersing it using a homogenizer under a pressure of 9.8 - 490 MPa (100 - 5,000 kgf/cm^2).

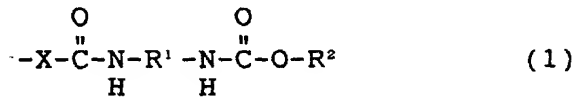
2. The process as claimed in claim 1, wherein the homogenizer is a high-pressure homogenizer.

3. The process as claimed in claim 1, wherein the homogenizer is a high-pressure homogenizer which operates so as to jet the swollen dispersion pressurized under a pressure of 9.8 - 490 MPa (100 - 5,000 kgf/cm^2) into a chamber from an orifice to disperse the swollen dispersion to treat it.

4. The process as claimed in any one of claims 1 to 3, wherein the aggregates of the hydrophobic group-containing polysaccharide have particle sizes of 10 - 30 nm and numbers of associations of the hydrophobic group-containing polysaccharide molecules of 3 - 20.

5. The process as claimed in any one of claims 1 to 4, wherein the hydrophobic group-containing polysaccharide has -XH groups (wherein X denotes oxygen atom or a nitrogen-containing group represented by NY with Y standing for hydrogen atom or a hydrocarbon group of 1 - 10 carbon atoms), wherein 0.1 - 10 -XH groups per 100 monosaccharide units constituting the

polysaccharide are replaced by one or more hydrophobic groups represented by the formula (1), namely,



(1)

in which X is the same as given above, R^1 denotes a hydrocarbon group having 1 - 50 carbon atoms and R^2 denotes a hydrocarbon group having 12 - 50 carbon atoms or a steryl.

6. The process as claimed in claim 5, wherein the polysaccharide to be substituted by hydrophobic group(s) consists of any one selected from the group consisting of pullulan, amylopectin, amylose, dextran, hydroxyethyl cellulose, hydroxyethyl dextran, mannan, levan, inulin, chitin, chitosan, xyloglucan and water-soluble cellulose.

ABSTRACT

A process for forming aggregates of hydrophobic group-containing polysaccharide in water, which comprises causing the hydrophobic group-containing polysaccharide to swell in water and treating the resulting swollen dispersion by dispersing it using a homogenizer under a pressure of 9.8 - 490 MPa (100 - 5,000 kgf/cm^2), whereby homogenous aggregates of the hydrophobic group-containing polysaccharide are formed in a simple and convenient way in large amount within a brief time.

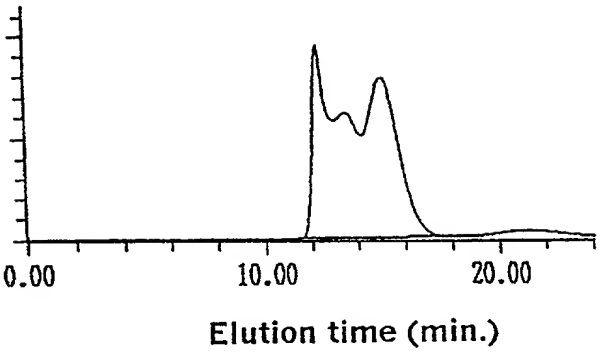
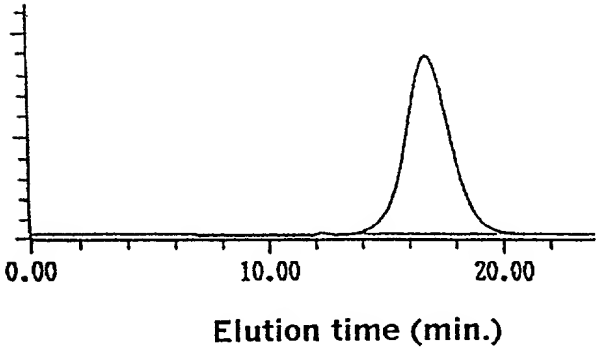

0.00
10.00
20.00
Elution time (min.)

Fig. 1 (a)


0.00
10.00
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Elution time (min.)

Fig. 1 (b)

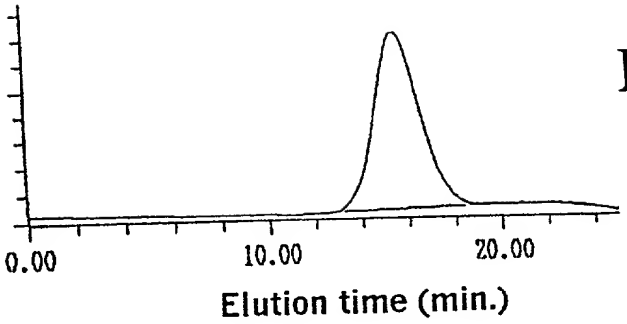


Fig. 2 (a)

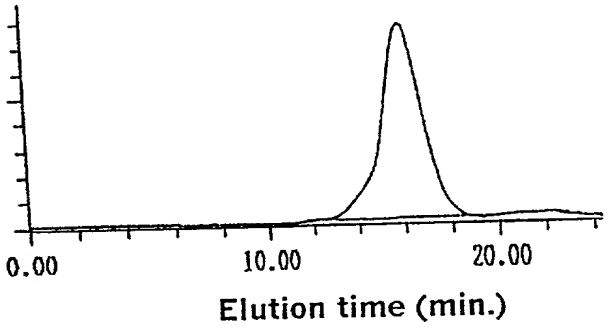


Fig. 2 (b)

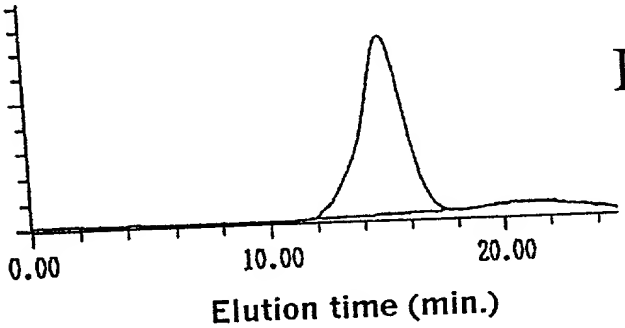


Fig. 2 (c)

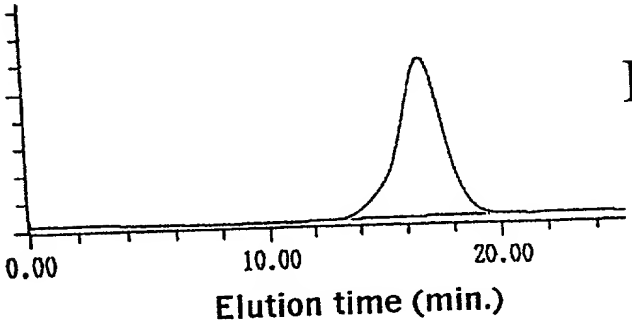


Fig. 2 (d)

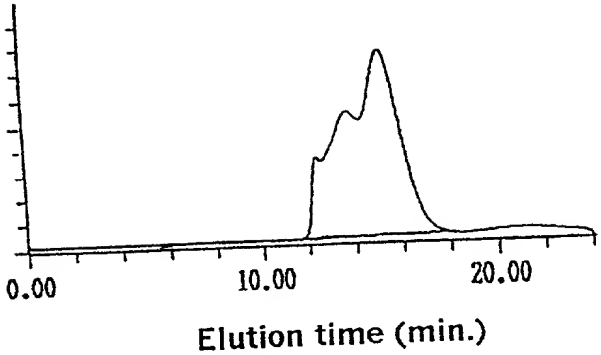


Fig. 3

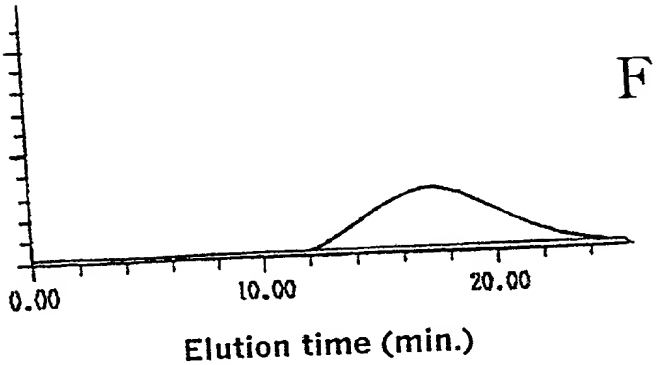


Fig. 4 (a)

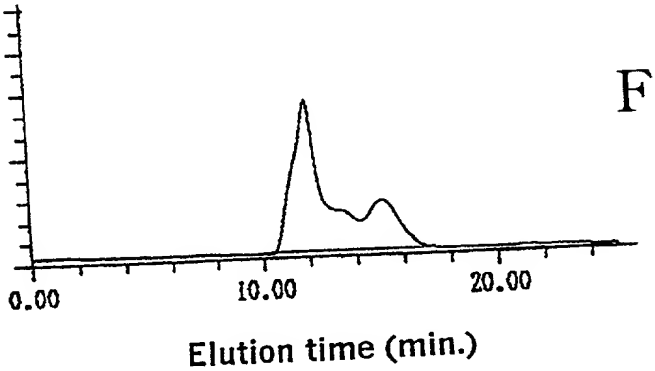


Fig. 4 (b)

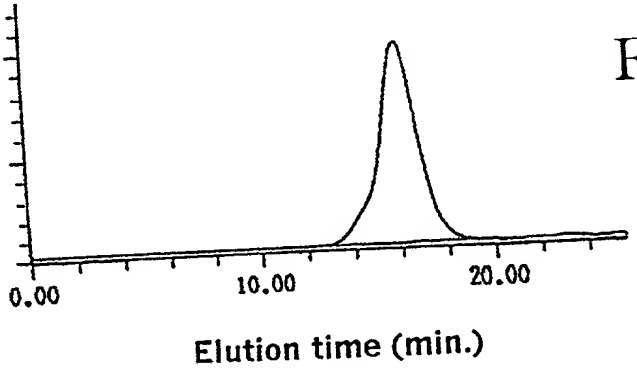
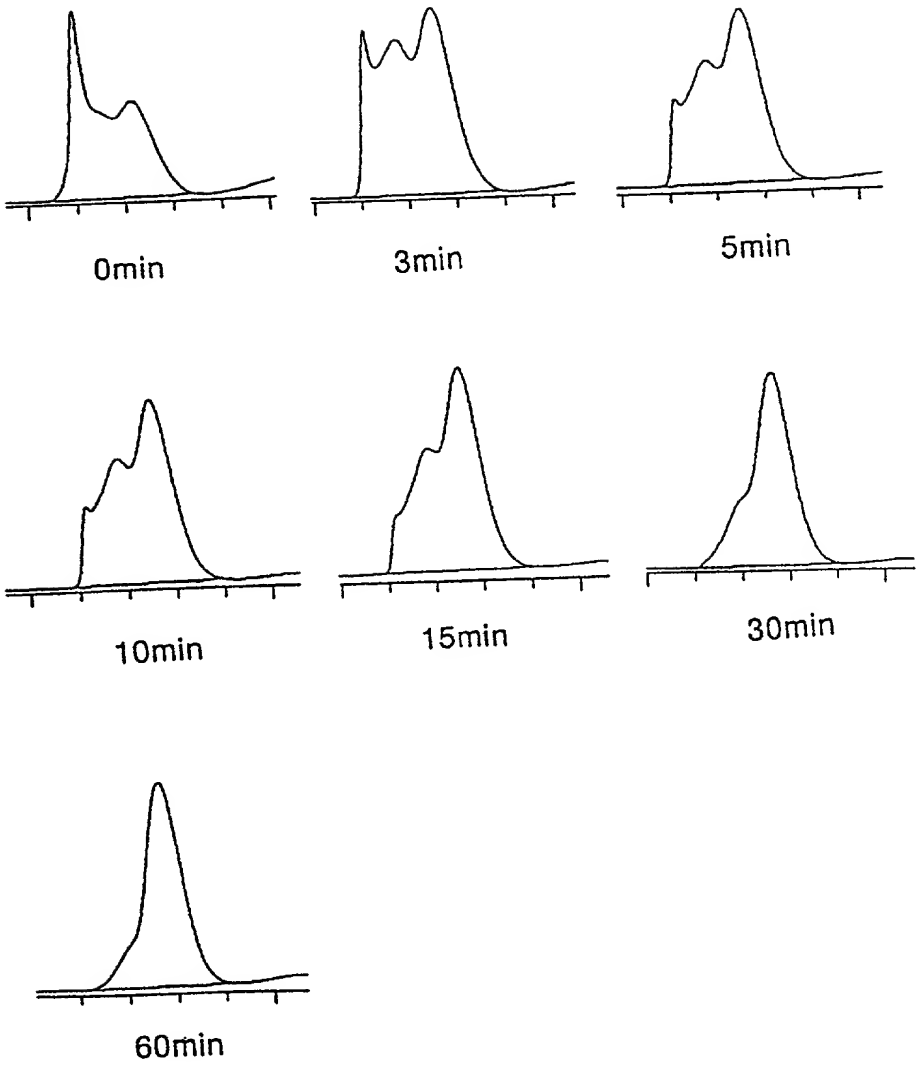
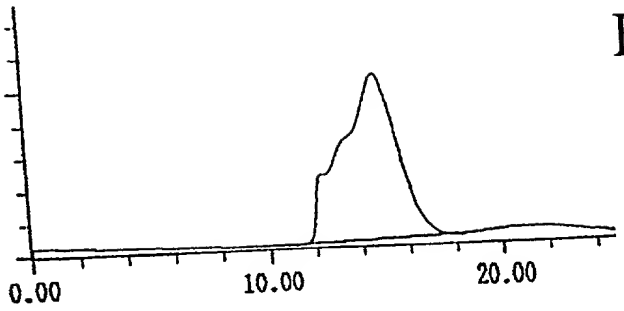


Fig. 4 (c)

Fig. 5

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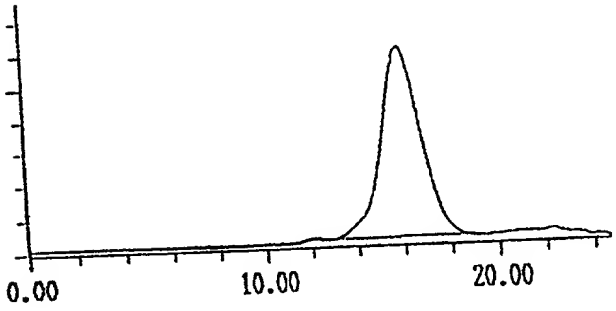
Elution time (min.)

Fig. 6 (a)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Elution time (min.)

Fig. 6 (b)

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled PROCESS FOR FORMING AGGREGATES OF HYDROPHOBIC GROUP-CONTAINING POLYSACCHARIDE, the

specification of which (check one) ___ is attached hereto. X was filed on March 31, 1999, as Application Serial No. PCT/JP 99/01684 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

5 Dale H. Thiel (Reg. No. 24 323), David G. Boutell (Reg. No. 25 072), Ronald J. Tanis (Reg. No. 22 724), Terryence F. Chapman (Reg. No. ~~32 549~~) and Mark L. Maki (Reg. No. 36 589).

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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